

## AMENDMENTS

### In the Specification:

Please replace the paragraph beginning at page 3, line 28, with the following rewritten paragraph:

--As described in Stea, A., *et al.* (1994) (*supra*), the  $\alpha_1$  subunits are generally of the order of 2000 amino acids in length, ranging from 1873 amino acids in  $\alpha_{1S}$  derived from rabbit to 2424 amino acids in  $\alpha_{1A}$  derived from rabbit. Generally, these subunits contain 4 internal homologous repeats (I-IV) each having six putative alpha helical membrane spanning segments (S1-S6) with one segment (S4) having positively charged residues every 3rd or 4th amino acid. There are a minority of a splice variant exceptions. Between domains II and III there is a cytoplasmic domain which is believed to mediate excitation-contraction coupling in  $\alpha_{1S}$  and which ranges from 100-400 amino acid residues among the subtypes. The domains I-IV make up roughly 2/3 of the molecule and the carboxy terminus adjacent to the S6 region of domain IV is believed to be on the intracellular side of the calcium channel. There is a consensus motif (QQ-E-L-GY-WI-E) (SEQ ID NO:44) in all of the subunits cloned and described in Stea, A., *et al.* (*supra*) downstream from the domain I S6 transmembrane segment that is a binding site for the  $\beta$  subunit.--

Please replace the paragraph beginning at page 6, line 7, with the following rewritten paragraph:

--Figure 6A-6E shows the nucleotide and deduced amino acid sequence of human T-type calcium channel  $\alpha_{1G}$ .--

Please replace the paragraph beginning at page 7, line 6, with the following rewritten paragraph:

--One distinguishing feature of the  $\alpha 1G$ ,  $\alpha 1H$  and  $\alpha 1I$  T-type channels over other types of calcium channels and sodium channels is that the pore region (P-region) in each of the four structural domains contains a diagnostic amino acid sequence implicated in channel permeability. Figure 8 shows that the T-type channels contain the residues glutamate/glutamate/aspartate/aspartate (single letter amino acid code: EEDD (SEQ ID NO:45)) in the P-regions of domains I-IV. In contrast, figure 8 shows that in sodium (Na) channels the P-region of the four domains contains the residues: aspartate/glutamate/lysine/alanine (single letter amino acid code: DEKA (SEQ ID NO:46)), while high threshold calcium channels such as the L-type channel contain the residues: glutamate/glutamate/glutamate/glutamate (single letter amino acid code: EEEE (SEQ ID NO:47)). The  $\alpha 1G$ ,  $\alpha 1H$  and  $\alpha 1I$  T-type channels are also distinct in this region compared to other types of ion channels including the *C. elegans* C11D2.6 and C27F2.3 and the rat NIC-channel (Figure 8).--

Please replace the paragraph beginning at page 7, line 18, with the following rewritten paragraph:

--A second distinguishing characteristic of the  $\alpha 1G$ ,  $\alpha 1H$  and  $\alpha 1I$  T-type channels compared to other types of calcium channels is that they do not contain a  $\beta$  subunit binding consensus sequence in the cytoplasmic linker separating domains I and II. In contrast, all high threshold calcium channels contain a consensus sequence (single letter amino acid code: QQ-E--L-GY--WI---E) (SEQ ID NO:44) shown to physically interact with the calcium channel  $\beta$  subunit (Pragnell, M., De Waard, M., Mori, Y., Tanabe, T., Snutch, T.P. & Campbell, K.P., 1994, Nature 368:67-70). Thus it appears the presence of a  $\beta$  subunit does not modify activity, nor is its presence required.--

Please replace the paragraph beginning at page 8, line 5, with the following rewritten paragraph:

--Alternatively, the T-type  $\alpha 1$  subunit molecules can be defined by homology to the human and rat nucleotide and amino acid sequences described herein. Thus, T-type  $\alpha 1$  subunits

will typically have at least 50%, preferably 70% homology in terms of amino acid sequence or encoding nucleotide sequence to the sequences set forth in SEQ ID NOS. 23-28 herein or those shown in Figure 6A-6E. Preferably, the homology will be at least 80%, more preferably 90%, and most preferably 95%, 97%, 98% or 99%.--

Please replace the paragraph beginning at page 8, line 11, with the following rewritten paragraph:

--Relative homology may also be defined in terms of specific regions; as set forth above, certain regions of T-type channel  $\alpha_1$  subunits have very high homologies while other regions, such as the cytoplasmic region between domains II and III have less homology. Thus, T-type  $\alpha_1$  subunits will have over 75% homology; preferably over 85% or over 95% homology, more preferably over 98% homology in domains I-IV to those of SEQ. ID. NOS. 23-28 or Figure 6A-6E. The degree of homology in the cytoplasmic region between domains II and III may be substantially less, *e.g.*, only 25% homology, preferably, 50% homology or more preferably 60% homology. Similarly, the intracellular region downstream of domain IV may be less homologous than within domains I-IV.--

Please replace the paragraph beginning at page 11, line 24, with the following rewritten paragraph:

--Following these protocols, full length mammalian  $\alpha_{1G}$ ,  $\alpha_{1H}$  and  $\alpha_{1I}$  calcium channel subunit cDNAs were isolated by using the 567 base pair human fragment (SEQ. ID NO. 19) to screen a rat brain cDNA library. Sequencing of the recovered sequences identified the three distinct classes of calcium channel subunits which have been denominated herein as  $\alpha_{1G}$ ,  $\alpha_{1H}$  and  $\alpha_{1I}$  subunits. For each class of subunit, complete sequencing of the largest cDNA confirmed that it represented only a portion of the predicted calcium channel coding region. Complete sequences for the three new subunits were obtained by rescreening the rat brain cDNA library with probes derived from the partial length cDNAs to obtain overlapping segments. These segments were combined to form a complete gene by restriction digestion and ligation. The

complete cDNA sequences of the rat  $\alpha_{1G}$ ,  $\alpha_{1H}$  and  $\alpha_{1I}$  subunits are given by SEQ. ID NOS. 23, 25 and 27, respectively. Corresponding amino acid sequences are given by SEQ. ID NOS. 24, 26 and 28. The same techniques are employed to recover human sequences by screening of a human or other mammalian library. Thus, for example, partial length human sequences for  $\alpha_{1G}$  and  $\alpha_{1H}$  T-type calcium channels have been recovered using the same probe (SEQ. ID NO. 19) and the full length rat  $\alpha_{1I}$  cDNA (SEQ. ID. NO. 27) has been used to recover a partial length DNA encoding a human  $\alpha_{1I}$  T-type calcium channel. The DNA and amino acid sequences for these partial length human calcium channels are given by SEQ. ID NOS. 30-35. A complete coding sequence for human  $\alpha_{1G}$  was also obtained and is set forth, along with the deduced amino acid reference, in Figure 6A-6E.--

Please replace the paragraph beginning at page 22, line 17, with the following rewritten paragraph:

--The remaining region of the 3'  $\alpha_{1G}$  subunit cDNA was obtained using the PCR method on a human thalamus cDNA library with primers MD19-sense (5'GCG TGG AGC TCT TTG GAG 3') (SEQ ID NO:48) and G26- antisense (5' GCA CCC AGT GGA GAA AGG TG 3') (SEQ ID NO:49). The PCR protocol used was 94°C -30 sec, 58°C -30 sec, 72°C -30 sec for 25 cycles (Bio-rad Gene Cyclor). A cDNA fragment of 1617 bp was subcloned into p-Gem-T-Easy plasmid vector (Promega) and sequenced. The 3'PCR cDNA was identified as a human  $\alpha_{1G}$  subunit spanning from Domain IV-S5 to the carboxyl terminus including the stop codon.--

Please replace the paragraph beginning at page 22, line 28, with the following rewritten paragraph:

--The complete nucleotide and amino acid sequences are shown in Figure 6A-6E.--